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Roose, J., & Pakrasi, H. (2008). The Psb27 protein facilitates manganese cluster assembly in photosystem II. *Journal of Biological Chemistry*, 283 (7), 4044-4050. <https://doi.org/10.1074/jbc.M708960200>

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The Psb27 Protein Facilitates Manganese Cluster Assembly in Photosystem II^{*S}

Received for publication, October 31, 2007, and in revised form, December 14, 2007 Published, JBC Papers in Press, December 18, 2007, DOI 10.1074/jbc.M708960200

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Photosystem II (PSII) is a large membrane protein complex that uses light energy to convert water to molecular oxygen. This enzyme undergoes an intricate assembly process to ensure accurate and efficient positioning of its many components. It has been proposed that the Psb27 protein, a luminal extrinsic subunit, serves as a PSII assembly factor. Using a *psb27* genetic deletion strain ($\Delta psb27$) of the cyanobacterium *Synechocystis* sp. PCC 6803, we have defined the role of the Psb27 protein in PSII biogenesis. While the Psb27 protein was not essential for photosynthetic activity, various PSII assembly assays revealed that the $\Delta psb27$ mutant was defective in integration of the $Mn_4Ca_1Cl_x$ cluster, the catalytic core of the oxygen-evolving machinery within the PSII complex. The other luminal extrinsic proteins (PsbO, PsbU, PsbV, and PsbQ) are key components of the fully assembled PSII complex and are important for the water oxidation reaction, but we propose that the Psb27 protein has a distinct function separate from these subunits. We show that the Psb27 protein facilitates $Mn_4Ca_1Cl_x$ cluster assembly in PSII at least in part by preventing the premature association of the other extrinsic proteins. Thus, we propose an exchange of luminal subunits and cofactors during PSII assembly, in that the Psb27 protein is replaced by the other extrinsic proteins upon assembly of the $Mn_4Ca_1Cl_x$ cluster. Furthermore, we show that the Psb27 protein provides a selective advantage for cyanobacterial cells under conditions such as nutrient deprivation where $Mn_4Ca_1Cl_x$ cluster assembly efficiency is critical for survival.

Photosystem II (PSII),³ a component of the photosynthetic electron transfer chain located in the thylakoid membranes of plants and cyanobacteria, catalyzes the light-driven conversion of water to molecular oxygen. This important reaction connects solar energy to the chemical energy used by the vast majority of life on earth and generates molecular oxygen as a byproduct to sustain the aerobic atmosphere of the planet. PSII is a membrane protein complex comprised of more than 20

protein subunits and numerous cofactors. A number of recent structural studies have increased our understanding of the architecture of the functional complex and provided new insights into the mechanism of this important enzyme (1–4). Briefly, photons initiate the transfer of electrons from a pair of chlorophylls at the reaction center through a series of cofactors to a membrane-soluble quinone. To reset the PSII reaction center, a catalytic center of $Mn_4Ca_1Cl_x$ removes electrons from water, yielding molecular oxygen.

The electron transfer reactions through PSII require precise positioning of its redox active cofactors. Proper assembly of the numerous PSII components is essential, and the PSII biogenesis pathway entails an ordered assembly of the constituent proteins and cofactors (5, 6). Moreover, PSII assembly occurs frequently because this enzyme continually undergoes a cycle of damage and repair. As a consequence of its normal function, the D1 membrane protein of PSII is irreversibly damaged and must be replaced with a newly synthesized copy (reviewed in Refs. 7 and 8). During this repair cycle, the complex is at least partially disassembled as the damaged D1 protein is proteolytically removed. Key events in the reassembly pathway include (1) the integration of a newly translated precursor D1 protein (pD1) with a C-terminal amino acid extension, (2) cleavage of the pD1 protein extension by the CtpA protease to yield the mature D1 protein, (3) assembly of the catalytic $Mn_4Ca_1Cl_x$ cluster, and (4) binding of a number of extrinsic proteins on the luminal side of the complex (reviewed in Ref. 9).

Clearly, PSII is a dynamic protein complex, and elucidation of the details of the PSII assembly pathway has been the focus of intense research. Many studies have been directed at defining the order of association of various PSII protein subunits (6, 8). Additionally, several PSII assembly factors have been identified using a variety of approaches and model organisms; examples include PratA, Slr2013, Slr0286, HCF136, Lpa1, and Lpa2 (10–15). Although these proteins are not components of the functional PSII enzyme, they assist the assembly of such PSII complexes. In the absence of these factors, PSII assembly is adversely affected.

Psb27, a component of the PSII complex, has also been hypothesized to play a role in PSII assembly (reviewed in Ref. 16). This small (11-kDa) protein has been shown to localize to the thylakoid lumen in cyanobacteria and plants (17, 18). Psb27 has also been shown to co-purify with PSII complexes in a number of different biochemical preparations (19–22). Sequence and biochemical analysis corroborates that the Psb27 protein is extrinsically associated with the luminal side of the PSII complex. However, in cyanobacteria it is tightly associated with the thylakoid membrane via an N-terminal lipid modification (21).

^{*} This work was supported in part by the National Science Foundation (MCB 0215359). The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

^S The on-line version of this article (available at <http://www.jbc.org>) contains supplemental Fig. S1.

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³ The abbreviations used are: PSII, Photosystem II; WT, wild type; Chl, chlorophyll; F_v/F_m , variable fluorescence yield; HA, hydroxylamine.

Several biochemical studies in cyanobacteria have demonstrated that the Psb27 protein associates with PSII pre-complexes that are incapable of evolving oxygen (20–22). These inactive pre-complexes lack manganese atoms and the extrinsic proteins PsbO, PsbU, PsbV, and PsbQ. In contrast, PSII complexes purified using a histidine-tagged version of the extrinsic protein PsbQ are highly active for oxygen evolution and lack the Psb27 protein (23). Thus, there appears to be an exchange of the Psb27 protein for the other extrinsic proteins on the luminal side of the PSII complex during the assembly of the oxygen-evolving machinery. Chen *et al.* (24) have previously reported that the Psb27 protein is necessary for efficient PSII repair in the vascular plant *Arabidopsis*. In the current study, we have used a genetic deletion mutant of the *psb27* (*slr1645*) gene in the cyanobacterium *Synechocystis* sp. PCC 6803 to more precisely define the function of the Psb27 protein. We report that the Psb27 protein is required for optimal PSII assembly in cyanobacteria. Furthermore, we show that the Psb27 protein specifically facilitates assembly of the $\text{Mn}_4\text{Ca}_1\text{Cl}_x$ cluster in PSII by preventing the premature binding of the other PSII extrinsic subunits.

EXPERIMENTAL PROCEDURES

Culture Conditions—Wild type (WT), Δpsb27 , ΔpsbO , and ΔpsbO , Δpsb27 strains of *Synechocystis* 6803 were grown in BG11 medium (25) at 30 °C under 30 $\mu\text{mol photons/m}^2\text{s}$. Δpsb27 , ΔpsbO , and ΔpsbO , Δpsb27 cells were grown in BG11 medium supplemented with 10 $\mu\text{g/ml}$ chloramphenicol, 10 $\mu\text{g/ml}$ spectinomycin, and 10 $\mu\text{g/ml}$ chloramphenicol plus 10 $\mu\text{g/ml}$ spectinomycin, respectively. To assess photoautotrophic growth, cells were diluted to an OD at 730 nm = 0.05 in BG11 medium and grown at 30 °C with shaking under 30 $\mu\text{mol photons/m}^2\text{s}$. The OD at 730 nm was measured every 24 h on a μQuant Microplate spectrophotometer (Biotek Instruments, Inc., Winooski, VT).

Mutant Strain Generation—The construction of the ΔpsbO strain used in this study has been described previously (26). For the Δpsb27 mutant, the region surrounding *slr1645* (*psb27*) was amplified from genomic DNA and replacement of the coding region of the gene with a chloramphenicol resistance cassette was achieved by using a fusion PCR technique (supplemental Fig. 1) (27, 28). Table 1 provides the primer sequences used in this method. The resulting fusion PCR product was used to transform WT *Synechocystis* cells. The double mutant ΔpsbO , Δpsb27 was generated by transforming the ΔpsbO strain with the Δpsb27 PCR product. Complete segregation of all strains was confirmed by PCR analysis of each locus.

Oxygen Evolution—Oxygen evolution activity was measured on a Clark-type electrode in the presence of artificial electron acceptors (1 mM potassium ferricyanide and 0.5 mM 2,6-dichloro-*p*-benzoquinone. Cells were diluted to 5 μg of Chl/ml in BG11 medium. The light intensity was adjusted between 237 and 8250 $\mu\text{mol photons/m}^2\text{s}$ using neutral density filters.

Spectrophotometric Assays—Chl *a* concentration was determined spectrophotometrically after extraction with methanol (29).

Photoinhibition and Recovery—To assay high light photoinhibition of PSII in the presence of the protein synthesis inhibi-

tor lincomycin, WT and Δpsb27 cells were diluted to 3 μg of Chl/ml in BG11 medium and subjected to 200 $\mu\text{mol photons/m}^2\text{s}$ of monochromatic red (627 nm) and blue light (455 nm) (equivalent to ~ 1000 $\mu\text{mol photons/m}^2\text{s}$ white incandescent light) from an LED light source (PSI, Brno, Czech Republic) with air bubbling at 30 °C in the presence of 20 $\mu\text{g/ml}$ of lincomycin. To measure the recovery of PSII activity after photoinhibition, cells were washed twice with fresh BG11 to remove the lincomycin and allowed to recover under non-damaging light intensity (50 $\mu\text{mol photons/m}^2\text{s}$, equivalent to ~ 100 $\mu\text{mol photons/m}^2\text{s}$ white incandescent light). Variable fluorescence yield (F_v/F_m) was monitored throughout photoinhibition and recovery using an FL-200 fluorometer (PSI).

Photoactivation Assays—Hydroxylamine (HA) treatment and photoactivation of cells were performed as described in Refs. 30 and 31. Specifically, WT and Δpsb27 cells were treated with HA to disassemble their manganese clusters. Late log phase cells were harvested and suspended in HN buffer (10 mM Hepes and 30 mM NaCl at pH 7.0) to a concentration of 200 μg of Chl/ml. These cells were incubated with 2 mM HA for 10 min. This and all subsequent steps prior to the photoactivation assay were performed in darkness. The HA-treated cells were diluted with 10 ml of HN buffer and centrifuged at 25 °C and 10000 $\times g$ for 6 min. The cells were washed three times with 30 ml of HNMC buffer (10 mM Hepes, 30 mM NaCl, 1 mM CaCl_2 , and 50 μM MnCl_2 at pH 7.0). Finally, the cells were suspended in HNMC buffer at 200 μg of Chl/ml.

For photoactivation under continuous light conditions, WT and Δpsb27 cells were treated with HA to remove the manganese clusters from PSII. Treated cells were kept in the dark prior to assay. Cells were then diluted to 5 μg of Chl/ml in HNMC buffer and incubated under photoactivation light at either 13, 67, or 129 $\mu\text{mol photons/m}^2\text{s}$ white light. After incubation at the indicated light intensity, oxygen evolution was measured at 8250 $\mu\text{mol photons/m}^2\text{s}$ in the presence of the electron acceptors 0.5 mM 2,6-dichloro-*p*-benzoquinone and 1 mM potassium ferricyanide on a Clark-type electrode.

For flash-induced photoactivation assays, HA-treated cells were deposited on a bare platinum electrode (Artisan Scientific), covered with an agarose disc of HNMC buffer, dark-incubated for 2 min, and subjected to a series of pre-flashes (0–2000) at 0.3 s and then a train of 20 measuring flashes at 0.3 s. The normalized average oxygen yield of flashes 16–20 for each measuring train is graphed as a function of pre-flashes.

Mixed Culture Assays—The mixed culture analyses of WT and Δpsb27 cells were performed essentially as described in Ref. 32. Cultures of WT and Δpsb27 cells were grown in 50 ml of BG11 to mid-exponential phase. Mixed cultures containing an equal number of WT and Δpsb27 cells were started and grown under 30 $\mu\text{mol photons/m}^2\text{s}$ in either BG11 or BG11 lacking CaCl_2 . The mixed culture was diluted to an OD at 730 nm = 0.05 every 48 h in fresh medium, and the remaining culture was used for total DNA extraction. Each mixed culture was subcultured a total of six times during the course of the experiment. PCR analysis of the *psb27* locus was performed with the segregation analysis primers Psb27E and Psb27F (Table 1) to determine the relative amounts of WT and Δpsb27 cells in the mixed cultures. The total DNA isolated from each subculture of the

Psb27 and PSII Manganese Cluster Assembly

TABLE 1

Primer sequences for $\Delta psb27$ mutant construction

The portions of the primers Psb27B and Psb27C corresponding to the chloramphenicol resistance marker are shown in *italics*.

Amplification of upstream region	Psb27A Psb27B	TACCTCAGTCAAAATCGCCC <i>CATGGTCATAGCTGTTTCCAGCCGTGACAACTGATTTTT</i>
Amplification of downstream region	Psb27C Psb27D	<i>ACTGGCCGTCGTTT</i> TACCTGGAATTATCCAGGCAGA ATGGTCGGCACTAGTTTTGG
Segregation analysis	Psb27E Psb27F	CCTGGCCAACTGGTAGGATA AGCCGGTCATAGGAAGGAGT

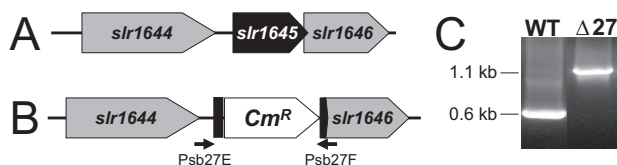


FIGURE 1. $\Delta psb27$ mutant. A, WT locus of the *psb27* (*slr1645*) gene. B, $\Delta psb27$ mutant locus in which the majority of the coding region of the *psb27* gene has been replaced by a chloramphenicol resistance marker (*Cm^R*). C, PCR segregation analysis of the $\Delta psb27$ mutant using the Psb27E and Psb27F primers (Table 1), whose positions are labeled in B.

mixed cultures was diluted to a final concentration of 20 ng/ μ L. The PCR reactions contained 10 ng of template DNA, 10 pmol of each primer, 250 μ M dNTP mixture, PCR buffer (50 mM Tris-HCl, pH 9.2, 16 mM ammonium sulfate, 2.5 mM MgCl₂, and 0.1% (w/v) Tween 20) and 1 unit of KlenTaq polymerase (W. Barnes, Washington University School of Medicine). PCR amplification involved 30 cycles at 94 °C for 30 s, 57 °C for 60 s, and 72 °C for 90 s. Agarose gels of the PCR products were visualized using the Kodak 1D Image Analysis software (Rochester, NY), and the relative amount of each product was quantified using the ImageJ software.

RESULTS

The Psb27 Protein Is Not Essential for Photosynthetic Activity in *Synechocystis* 6803—It has been shown previously that the Psb27 protein associates with PSII pre-complexes that cannot evolve oxygen (20–22). Furthermore, analysis of an *Arabidopsis* mutant line lacking the Psb27 protein indicated that this protein plays a role in efficient reassembly of PSII after light-induced damage (24). To more precisely define the role of Psb27 in PSII biogenesis and function, a genetic deletion of the *psb27* (*slr1645*) gene in the cyanobacterium *Synechocystis* 6803 was constructed (supplemental Fig. S1). In this $\Delta psb27$ mutant, the majority of the coding sequence of the *psb27* gene has been replaced by a chloramphenicol resistance gene (Fig. 1). Because *Synechocystis* 6803 cells usually contain multiple copies of their genome, PCR analysis of the *psb27* locus was used to confirm complete segregation of the $\Delta psb27$ mutant allele (Fig. 1C).

To assay for possible photosynthetic defects in the $\Delta psb27$ mutant, photoautotrophic growth and oxygen-evolving activity were measured. The $\Delta psb27$ mutant grew at the same rate as WT cells in BG11 medium (Fig. 2A). WT and $\Delta psb27$ cells also displayed similar oxygen evolution rates at the light intensities measured (Fig. 2B). These results indicate that the Psb27 protein is not absolutely essential for photosynthetic activity in *Synechocystis* 6803 cells, similar to previously published observations for the *Arabidopsis* mutant lacking the Psb27 protein (24).

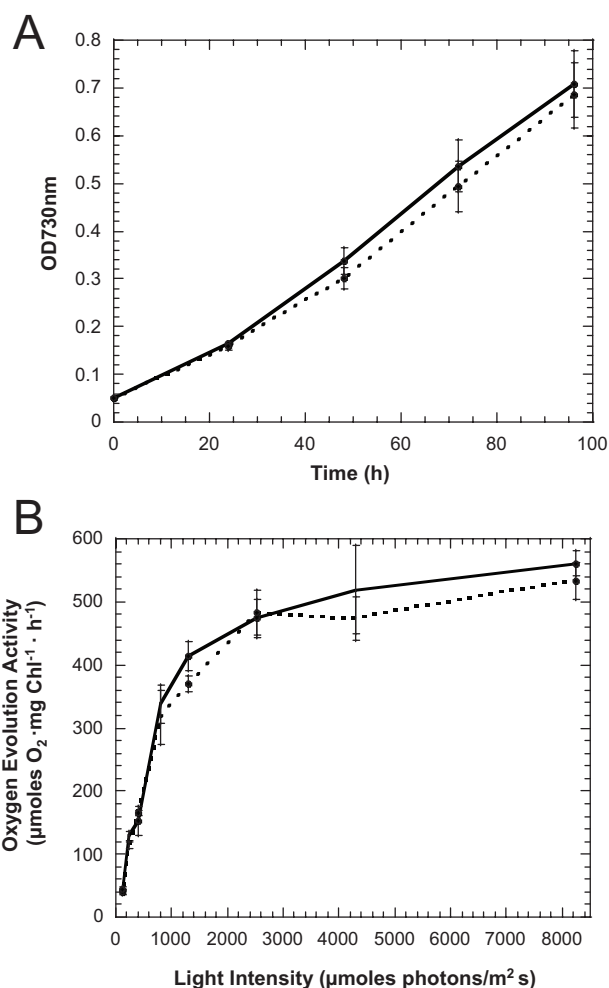


FIGURE 2. Photosynthetic competence of *Synechocystis* cells in BG11 medium. A, photoautotrophic growth of WT (solid line) and $\Delta psb27$ (dashed line) cells. Error bars represent S.E., $n = 3$. B, light saturation curves of oxygen evolution for WT (solid line) and $\Delta psb27$ (dashed line) cells. Error bars represent S.E., $n = 3$.

The Psb27 Protein Is Necessary for Efficient PSII Repair upon Photodamage—Although the Psb27 protein is not required for PSII activity, previous studies have indicated a possible role for Psb27 in PSII assembly or during the repair cycle (24). As described previously, the D1 protein within the PSII complex is damaged, resulting in loss of activity, and must be replaced with a newly synthesized copy to regenerate functional PSII complexes. This damage, termed photoinhibition, is induced by exposure to high light intensities. To determine whether the Psb27 protein plays a role in the PSII damage and repair cycle in *Synechocystis*, high light photoinhibition and recovery of PSII activity were measured in WT and $\Delta psb27$ cells.

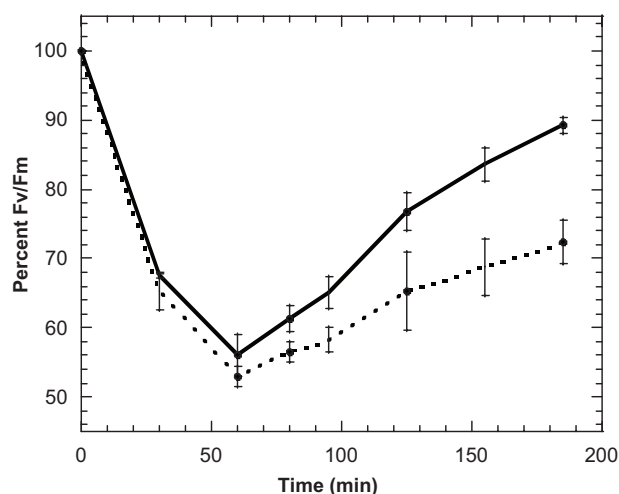


FIGURE 3. **High light photoinhibition and recovery.** WT (solid line) and $\Delta psb27$ (dashed line) cells were incubated under high light in the presence of the protein synthesis inhibitor lincomycin for 1 h. Cells were then washed twice with fresh medium to remove the lincomycin and then incubated at a lower non-inhibiting light intensity. Variable fluorescence yield (F_v/F_m) was measured throughout the experiment. Error bars represent S.E., $n = 3$.

To monitor PSII photoinhibition and recovery, WT and $\Delta psb27$ cells were subjected to high light illumination in the presence of the protein synthesis inhibitor lincomycin and PSII activity was monitored as the variable fluorescence yield (F_v/F_m) throughout the experiment. To assay recovery after the photoinhibition treatment, the lincomycin was removed and cells were incubated under a lower non-damaging light intensity to allow for reassembly of functional PSII complexes (Fig. 3). WT and $\Delta psb27$ cells showed a similar decrease in F_v/F_m upon high light treatment in the presence of lincomycin. Thus, PSII stability in the $\Delta psb27$ mutant was not significantly different from that of WT cells. However, the $\Delta psb27$ cells did not recover after photoinhibition to the same extent as WT cells. Therefore, the Psb27 protein is necessary for efficient PSII repair.

The Psb27 Protein Specifically Functions in PSII Manganese Cluster Assembly—To probe a more specific role of Psb27 during PSII assembly, we tested the efficiency of manganese cluster assembly in WT and $\Delta psb27$ cells. Assembly of the manganese cluster into PSII complexes is a multistep process, termed photoactivation, that involves sequential binding and photo-oxidation of manganese atoms, interspersed with one dark rearrangement step and the incorporation of Ca^{2+} and Cl^- ions to yield the functional catalytic $Mn_4Ca_1Cl_x$ core of the oxygen-evolving machinery (reviewed in Ref. 9). To assay manganese cluster assembly *in vivo*, cells were treated with the small reductant HA to release the manganese clusters from functional PSII complexes and then illuminated in the absence of HA to allow for reassembly of functional manganese clusters capable of oxygen evolution.

HA-treated WT and $\Delta psb27$ cells were photoactivated using continuous white light of varying intensity (Fig. 4). In these assays, WT and $\Delta psb27$ cells showed similar photoactivation kinetics under the lowest light intensity (Fig. 4A). However, upon incubation under higher light intensities, $\Delta psb27$ cells failed to assemble manganese clusters to the extent observed in

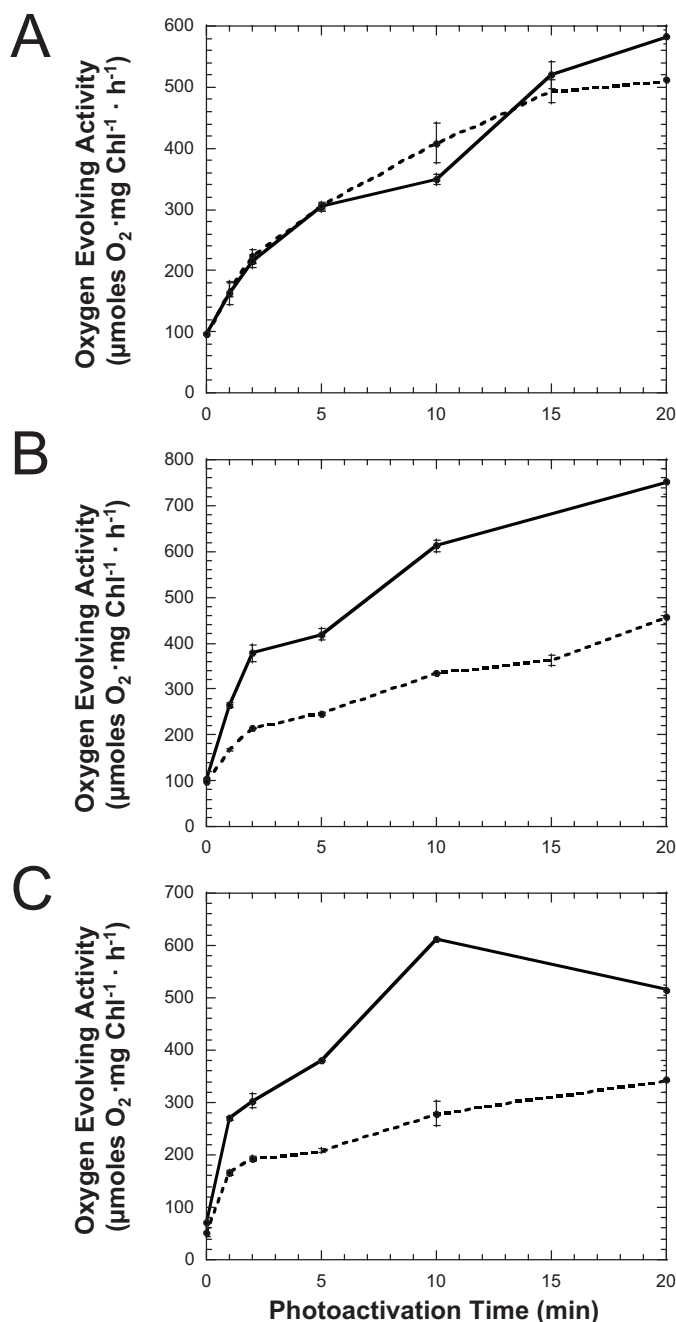


FIGURE 4. **Photoactivation under continuous light.** HA-treated WT (solid lines) and $\Delta psb27$ (dashed lines) cells photoactivated at 13 (A), 67 (B), and 129 μmol photons/ $m^2 \cdot s$ (C) white light. Cells were incubated for the indicated times under the respective photoactivation light intensity, and then oxygen evolution activity was measured as described under "Experimental Procedures." Error bars represent S.E., $n = 3$.

WT cells (Fig. 4, B and C). These results indicate that the Psb27 protein specifically facilitates manganese cluster assembly in PSII and its role is more critical under higher light intensities. Although the manganese cluster assembly process requires light, light can also result in damage to the PSII complexes. Moreover, photoinhibition is especially damaging to manganese-depleted PSII complexes (33, 34). Therefore, photoactivation and photoinhibition are competing processes during the manganese cluster assembly assays. WT cells, which are very efficient in manganese cluster assembly, are less susceptible to

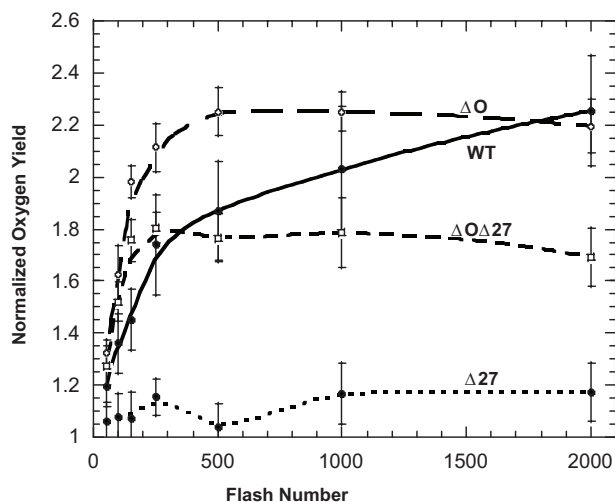


FIGURE 5. Double mutant photoactivation as a function of flash number. HA-treated WT (solid line, closed circles), $\Delta psb27$ (dashed line, closed circles), $\Delta psbO$ (dashed line, open circles), and $\Delta psbO, \Delta psb27$ (dashed line, open squares) cells were subjected to a series of flashes on a bare platinum electrode. The normalized average oxygen yield of flashes 16–20 for each measuring train is graphed as a function of the number of photoactivating preflashes. Error bars represent S.E., $n = 3$.

photoinhibition, whereas the $\Delta psb27$ cells, which are less efficient at manganese cluster assembly, are more prone to photoinhibition. For the $\Delta psb27$ mutant, photoinhibition predominates at higher light intensities in manganese-depleted cells, resulting in lower recovery of oxygen-evolving activity.

The Psb27 Protein Excludes Other Extrinsic Proteins during Manganese Cluster Assembly—Several roles for the Psb27 protein have been postulated. Among them is the role of this protein as a metal delivery chaperone to PSII. This scenario is unlikely because the Psb27 protein lacks any known metal binding motifs and previous studies reported that Psb27-associated PSII complexes lack manganese atoms. Another hypothesis is that the Psb27 protein may facilitate manganese cluster assembly into PSII by inducing an optimal conformation of the luminal side of the complex. This may also involve exclusion of the extrinsic proteins (PsbO, PsbU, PsbV, and PsbQ) normally associated with active PSII complexes as their premature binding may act as a hindrance to the incoming manganese atoms. In fact, the PsbO protein has been described as a barrier to the PSII manganese binding sites (35), and $\Delta psbO$ mutant cells show an increase in photoactivation efficiency compared with WT cells (30).

To more precisely assay the efficiency of manganese cluster assembly in PSII, a different photoactivation assay was used. HA-treated cells were subjected to a series of intense flashes from a Xenon lamp, and the resultant oxygen yield measured on a bare platinum electrode was used as a gauge of assembled active PSII centers. If a strain is more efficient at manganese cluster assembly, then a reduced number of flashes will be required to produce the saturating oxygen yield. WT cells displayed a typical photoactivation curve, in that with increasing flash number WT cells were able to assemble more functional manganese clusters in PSII, resulting in an increase in oxygen yield (Fig. 5). The $\Delta psbO$ mutant had a faster kinetics in manganese cluster assembly as published previously (30). In contrast, the $\Delta psb27$ cells did not reassemble manganese clusters

during this assay (Fig. 5). The difference in the ability of $\Delta psb27$ cells to assemble manganese clusters in this assay relative to the previous assay is likely due to the difference in photoactivation light (continuous versus intense flashes) used in the two assays. Clearly, $\Delta psb27$ cells were unable to effectively use the discrete flashes produced by the xenon flash lamp. Overall, the manganese cluster assembly phenotype of $\Delta psb27$ cells is strikingly different from that of the previously characterized PSII extrinsic protein mutants such as $\Delta psbO$.

If the Psb27 protein facilitates manganese cluster assembly by preventing the premature binding of other extrinsic proteins such as PsbO, then we predict that manganese cluster assembly would be more efficient in the double mutant $\Delta psbO, \Delta psb27$ than the $\Delta psb27$ mutant. In other words, if the Psb27 protein normally functions to exclude proteins such as PsbO, the genetic removal of the *psbO* gene in the $\Delta psb27$ mutant background should restore a more normal manganese cluster assembly phenotype. To test this hypothesis, manganese cluster assembly was also assayed in the double mutant $\Delta psbO, \Delta psb27$ (Fig. 5). Indeed, the double mutant $\Delta psbO, \Delta psb27$ was capable of photoactivation under these conditions whereas the $\Delta psb27$ mutant was not (Fig. 5). This result implies that one function of the Psb27 protein is to exclude PsbO, and presumably also the other extrinsic proteins, until the manganese cluster is assembled.

The Psb27 Protein Provides a Selective Advantage for Cellular Growth—The experiments outlined in this study indicate that Psb27 is necessary for efficient PSII repair after photodamage and has a specific role in the assembly of the manganese cluster. However, under a variety of culture conditions and during other photosynthesis assays, the $\Delta psb27$ mutant was not measurably different from WT yet the *psb27* gene is found in the vast majority of oxygenic photosynthetic organisms (16). Hence, it is reasonable to expect that the presence of the Psb27 protein benefits these organisms during their growth in nature, where deprivation of key nutrients and fluctuating light conditions are often encountered. In contrast, the commonly used laboratory conditions for organisms such as *Synechocystis* consist of unnaturally high concentrations of nutrients.

To evaluate the effect of Psb27 on organismal fitness, mixed culture experiments with WT and $\Delta psb27$ cells were performed. In these experiments, a mixed culture inoculated with an equal number of WT and $\Delta psb27$ cells was grown under different conditions and samples were taken to determine the relative amounts of each strain over a time course. If the absence of the Psb27 protein reduces the fitness of the organism, then the WT cells should overgrow the $\Delta psb27$ cells in such a mixed culture. During growth in BG11, the relative amounts of the two strains remained unaltered during the entire time course of the experiment (Fig. 6). In contrast, during growth in a medium lacking $CaCl_2$, the $\Delta psb27$ cells were completely out-competed by WT cells after four rounds of subculturing and the PCR band corresponding to the $\Delta psb27$ locus could not be detected (Fig. 6). In medium lacking $CaCl_2$, manganese cluster assembly must occur with optimal efficiency because calcium and chloride, two key ionic cofactors, are limiting. Therefore, the Psb27 protein provides a significant benefit to cells under conditions where

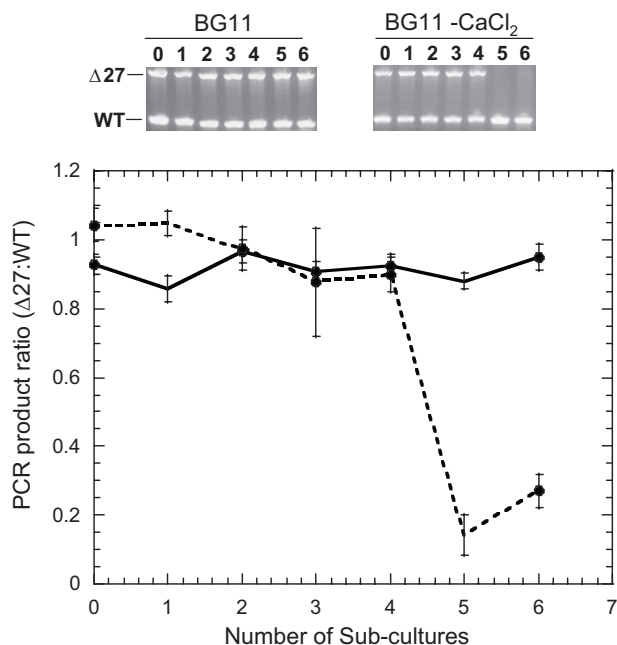


FIGURE 6. **Mixed culture competition experiment.** PCR products obtained from the mixed cultures grown under the indicated conditions over six sub-cultures of the original mixed culture. These products correspond to the *psb27* locus in WT and $\Delta psb27$ cells as in Fig. 1. The graph shows the ratio of the $\Delta psb27$ and WT PCR products over six sub-cultures of the mixed culture for the different growth conditions: BG11 (solid line) and BG11-CaCl₂ (dashed line). Error bars represent S.E., n = 4.

efficient manganese cluster assembly is important for survival.

DISCUSSION

It has been previously hypothesized that the Psb27 protein functions in the PSII assembly pathway, but the precise step was unknown. In this work, we used a genetic deletion mutant in the cyanobacterium *Synechocystis* 6803 to more precisely define the function of Psb27. When grown in nutrient-rich BG11 medium, the *Synechocystis* $\Delta psb27$ mutant was similar to WT with respect to photoautotrophic growth and photosynthetic activity. However, the recovery of the $\Delta psb27$ mutant after photoinhibition was slower relative to WT cells, in agreement with observations published for the *Arabidopsis* mutant (24). Altogether, the results of this work and previous studies indicate that the Psb27 protein assists in the reassembly of functional PSII complexes after photodamage.

The PSII repair process entails multiple steps such as partial disassembly to remove the damaged D1 protein followed by reassembly of the manganese cluster and binding of the extrinsic proteins PsbO, PsbU, PsbV, and PsbQ in cyanobacteria. A deficiency in any of these events would result in slower recovery from photoinhibition. Two lines of evidence from previous studies suggested that the Psb27 protein functions during PSII reassembly rather than disassembly of damaged PSII complexes: (a) the degradation of the damaged D1 protein was not affected in the *Arabidopsis* *psb27* mutant (24) and (b) ¹⁵N-labeling studies have indicated that the Psb27 protein is associated with PSII pre-complexes containing newly synthesized D1 protein (21). Therefore, to determine the specific function of

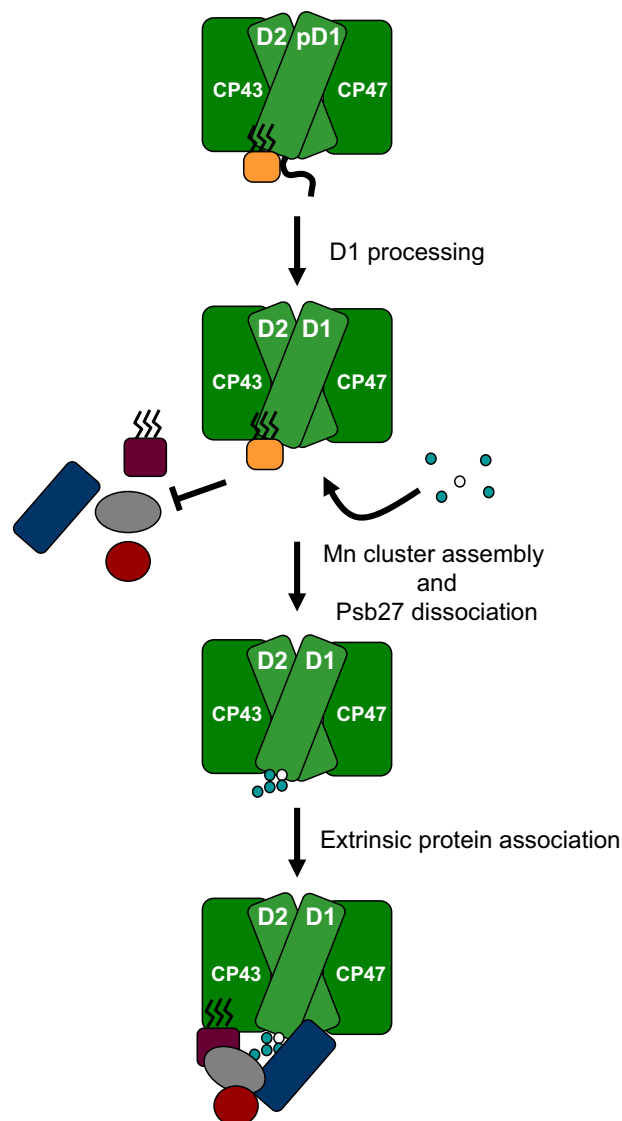


FIGURE 7. **Model for PSII assembly.** The core membrane proteins are shown in green. The extrinsic proteins are Psb27 (orange), PsbO (dark blue), PsbU (red), PsbV (gray), and PsbQ (purple). The Psb27 and PsbQ lipoproteins are depicted with their lipid modifications. The atoms of the manganese cluster are shown in blue and white. The assembly events are labeled. See "Discussion" for details.

the Psb27 protein, we focused on the assembly steps of the luminal side of PSII complexes.

In this study, we show that the Psb27 protein facilitates assembly of the PSII Mn₄Ca₁Cl_x cluster. The $\Delta psb27$ mutant exhibited significant defects during photoactivation assays. Previous biochemical studies have shown that the Psb27 protein is associated with PSII complexes that lack manganese (20–22). Conversely, purified PSII complexes that are highly active and contain stoichiometric amounts of manganese lack the Psb27 protein (23). Altogether, these data suggest that the Psb27 protein associates with PSII pre-complexes to facilitate integration of the Mn₄Ca₁Cl_x cluster and then dissociates from the complex. Another distinct characteristic of the Psb27-associated PSII complexes is the lack of the other extrinsic proteins PsbO, PsbU, PsbV, and PsbQ that are normally associated with active PSII complexes (20–22). We provide evidence that the

Psb27 protein aids the photoactivation process at least in part by excluding the other extrinsic proteins to allow for efficient access of the manganese, calcium, and chlorine atoms. Because the photoactivation process entails a number of steps and distinct intermediates, a more detailed analysis is necessary to evaluate the effect of Psb27 on the incorporation of the individual ionic cofactors as well as the specific kinetic steps during $Mn_4Ca_1Cl_x$ cluster assembly.

From these data along with those from previous studies, we propose the following sequence of events for the biogenesis of the lumenal side of PSII complexes (Fig. 7). The Psb27 protein associates with PSII pre-complexes early during the biogenesis pathway, perhaps even prior to the processing of the pD1 protein (20). The pD1 protein is processed by the CtpA protease to remove the C-terminal extension that protrudes from the lumenal side of the complex. Upon generation of the mature D1 protein, the $Mn_4Ca_1Cl_x$ cluster is assembled with the assistance of the Psb27 protein. Psb27 then dissociates to allow for the binding of the extrinsic proteins PsbO, PsbU, PsbV, and PsbQ. Note that these events in the assembly of the lumenal side of PSII are relevant for both *de novo* PSII synthesis and the PSII repair cycle after D1 photodamage.

Photosynthetic organisms daily experience fluctuations in light intensity and must survive in a variety of environments with different nutrient availability. The standard laboratory conditions that are routinely used in studies of *Synechocystis* mutants do not accurately reflect that of the natural environment. Thus, it may be difficult to interpret the significance of subtle phenotypic differences between WT and mutant cells. Under natural conditions where the concentrations of critical elements such as calcium and chlorine are low, the Psb27 protein provides a distinct benefit for cells by assisting optimal PSII assembly.

Acknowledgment—We thank members of the Pakrasi laboratory for many useful discussions.

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